

## ■ Biological Chemistry &amp; Chemical Biology

## Synthesis of New Aza- and Thia-Crown Ether Based Amino Acids

Tobias Schneider,<sup>[a]</sup> Nico Brüßow,<sup>[a]</sup> Alev Yuvanc,<sup>[a]</sup> and Nediljko Budisa<sup>\*[a, b]</sup>

Crown ethers have been known since the middle of the 20<sup>th</sup> century as metal chelating agents with numerous applications. For example, amino acids functionalized with crown ethers have been used to create artificial ion channels. However, only a few of those crown amino acids are known, all of which contain exclusively oxygen as heteroatoms in the crown ether moiety. Alternative structures may be desirable to explore new biological or pharmaceutical applications. Hence, we aim to expand the scope of available crown ether amino acids - in particular those with heteroatoms other than oxygen. Herein we describe the synthesis of three previously unknown amino acids bearing a crown ether moiety. We use two distinct synthetic routes to avoid the use of hazardous reagents. Two of these three amino acids bear sulfur atoms in the crown ether, while the third contains a nitrogen atom.

## Introduction

Crown ethers were first described in the mid-1960s, originally as an unwanted synthetic byproduct.<sup>[1]</sup> As a general feature they contain multiple  $-\text{CH}_2\text{CH}_2\text{O}-$  units, which are connected to form a circular molecule. However, many crown ethers derivatives are known in which oxygen is replaced by other heteroatoms, typically with either nitrogen or sulfur. Since their original discovery a variety of applications for crown ethers have been developed, mainly as metal chelators and phase transfer catalysts.<sup>[2]</sup> The flexible backbone and regular occurrence of atoms with available electron lone pairs allows for the formation of complexes with a multitude of metal ions as well

as other positively charged species like ammonium groups.<sup>[3]</sup> The affinity towards different cationic species is primarily determined by the ring size and type of heteroatoms.<sup>[4]</sup>

Interestingly, crown ethers have also been shown to have applications in a biological or medicinal context. For example, certain crown ether compounds show high antimicrobial activity.<sup>[5,6]</sup> Additionally, crown ethers show potential as anti-cancer agents and for other biomedical applications.<sup>[5,7]</sup> Furthermore, aromatic amino acids bearing crown ether moieties have been synthesized and were successfully used to create artificial ion channels.<sup>[8,9]</sup> The channels are formed by overlap of several crown ether moieties, which are held in place by connection to a  $\alpha$ -helical peptide backbone. These structures in particular have potential to be incorporated into proteins with additional domains. Thus, ion channels that open only under specific conditions might be created.

Until now, the incorporation of crown ether amino acids (CEAAs) into peptide scaffolds was achieved using solid phase peptide synthesis.<sup>[9]</sup> However, CEAAs may be suitable for direct incorporation into proteins by orthogonal translation techniques.<sup>[10]</sup> This could enable a direct *in vivo* production of larger proteins containing crown ether moieties, which might enable design of more complex artificial ion channels than would be accessible by solid phase synthesis.

Known CEAAs are based on the scaffold of the natural amino acid dihydroxyphenylalanine (DOPA). The free hydroxyl groups of DOPA are alkylated to form a  $\text{O}-\text{C}-\text{O}$  unit in the backbone of the crown ether moiety (Figure 1). However, the number of CEAAs present in literature is relatively small and limited to oxygen as heteroatom in the crown ether moiety. Therefore, our goal was to broaden the scope of available structures. In particular, we aimed to create novel CEAAs with heteroatoms other than oxygen to alter ion selectivity.

## Results and discussion

The published synthetic route towards DOPA derived oxygen-based crown ether amino acids involves a cyclization reaction on a protected DOPA analog using a crown ether precursor with two leaving groups (usually halides or sulfonate groups / see scheme 1).<sup>[11]</sup> The aromatic hydroxyl groups serve as nucleophiles and form part of the crown O atoms in the product.

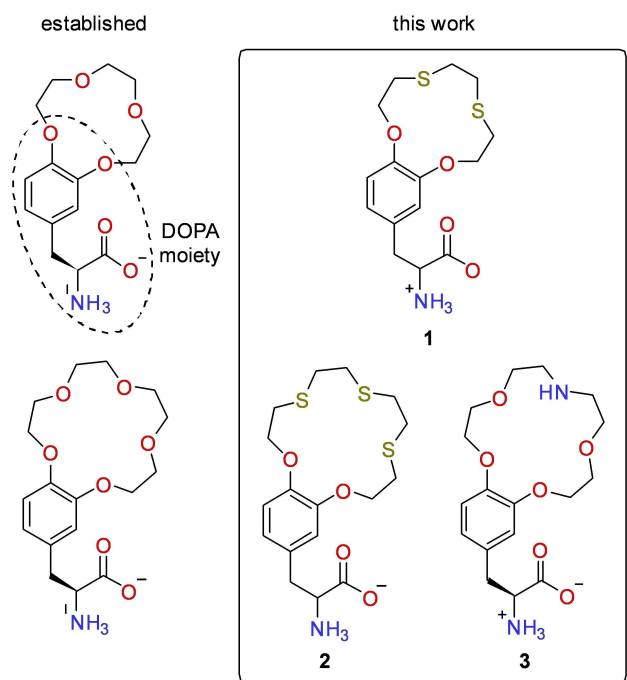
However, this synthetic route is impractical in the case of the sulfur- or nitrogen-containing crown ethers. In this case the electrophilic precursor molecule would require the presence of a sulfur atom at a carbon atom in the  $\beta$ -position to a leaving

[a] Dr. T. Schneider, N. Brüßow, A. Yuvanc, Prof. Dr. N. Budisa  
Institute of Chemistry  
Technical University of Berlin  
Müller-Breslau-Str. 10, Berlin, 10623, Germany  
E-mail: nediljko.budisa@tu-berlin.de

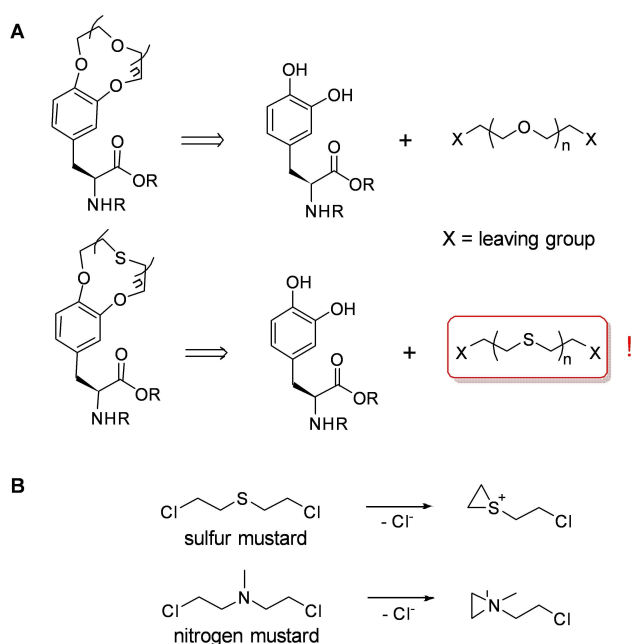
[b] Prof. Dr. N. Budisa  
Department of Chemistry  
University of Manitoba  
144 Dysart Rd, 360 Parker Building,  
R3T 2 N2 Winnipeg, MB, Canada  
E-mail: nediljko.budisa@umanitoba.ca

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**Figure 1.** Established crown ether amino acids and new crown ether amino acids 1–3 synthesized in this work



**Scheme 1.** A) Retrosynthetic approach for the synthesis of DOPA based CEAs. Using a similar approach to synthesize sulfur- or nitrogen-containing crown ethers would require harmful and reactive sulfur mustard analogs (red box). B) The undesirable reactivity of sulfur and nitrogen mustard analogs.

group (Scheme 1 red box). Unfortunately, this is a common feature of a class of chemical warfare reagents called mustard-gases.<sup>[12]</sup> In addition to causing second and third degree skin burns on contact, these compounds are known to be highly

toxic and carcinogenic.<sup>[13]</sup> These effects arise from the nucleophilic properties of a sulfur or nitrogen atom which lead to intramolecular ring formation. The thereby formed sulphonium or aziridinium intermediate is highly reactive and may alkylate biomolecules like DNA or other nucleophiles.<sup>[14]</sup>

Hence, this approach has two drawbacks in the case of sulfur or nitrogen containing crown ether moieties:

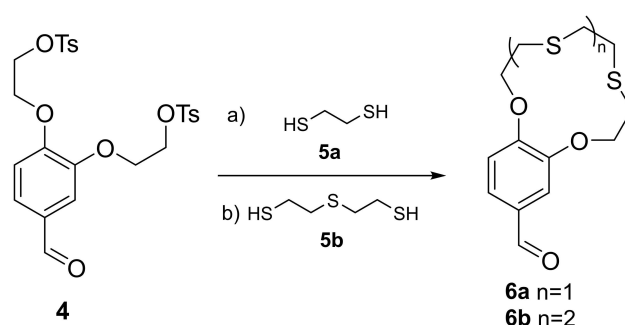
1. Formation of sulphonium or aziridinium ions may reduce stability of the reactant, especially if better leaving groups than chloride are used. Additionally, these cations would likely interfere with nucleophilic formation of the desired crown ether moiety.
2. Highly hazardous, and controlled, substances would have to be acquired, stored and used in reactions.

Hence, only few cases of cyclisation reactions with mustard-like electrophiles are published in literature.<sup>[15]</sup> Generally, thiols, amines or amides are preferred as nucleophiles to achieve ring formation in crown ether synthesis.<sup>[16]</sup> For optically active crown ethers with certain aromatic components like thiophenes, ring formation may be achieved by Glaser–Eglinton–Hay coupling and subsequent transformations.<sup>[17]</sup>

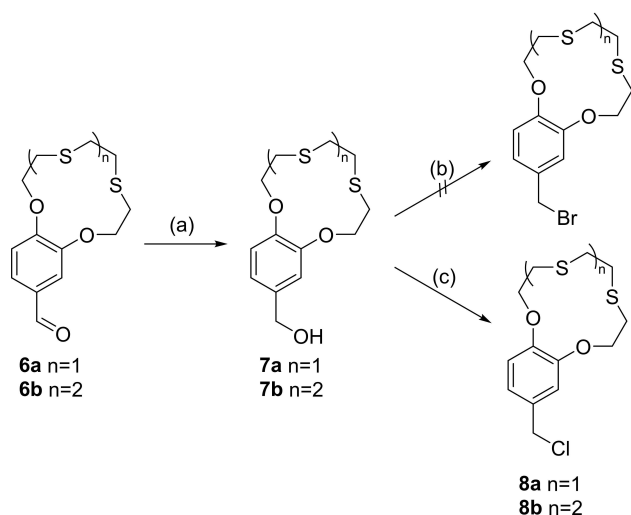
Due to these problems we decided to use an alternative approach to synthesize the sulfur containing CEAs 1 and 2. This approach is based on the use of thiol nucleophiles and the installation of leaving groups on a DOPA precursor 4 (see scheme 2). 4 was synthesized following a modified procedure described by Fedorova et al.<sup>[18]</sup> We used dithiols 5a/b to construct the thia crown ether moiety with a ring size of 12 (6a) and 15 (6b) atoms, respectively.

Aldehydes 6a/b were then reduced to the corresponding benzylic alcohols 7a/b. At first, we planned to convert 7a/b to the corresponding benzyl bromides for the purpose of consecutive alkylation. However, attempts to obtain benzylbromides with  $\text{PBr}_3$  as reagent gave low yields, possibly due to side reactions involving ring sulfur atoms. The conversion of 7a/b with  $\text{SOCl}_2$  to the corresponding benzyl chlorides 8a/b (scheme 3) was successful and gave excellent yields.

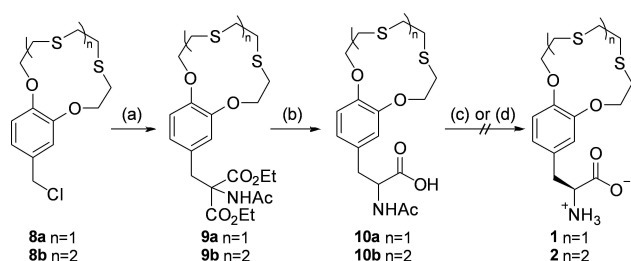
The chlorides 8a/b were then alkylated by diethylacetamido malonate (Scheme 4). The resulting *N*-Acetylmalonate esters 9a/b were obtained in acceptable yields. Following basic hydrolysis of the ethyl esters, the acidic decarboxylation gave the *N*-acetyl amino acids 10a/b. However, neither treatment



**Scheme 2.** Synthesis of thia benzo-crown ethers 6a and 6b. Reagents and conditions: (a) and (b)  $\text{Cs}_2\text{CO}_3$ , MeCN, 46% / 40%



**Scheme 3.** Synthesis of intermediates. Reagents and conditions: (a) NaBH<sub>4</sub>, EtOH, THF, **7a** 96%; **7b** 95% (b) PBr<sub>3</sub>, DCM (c) SOCl<sub>2</sub>, DCM, pyridine, **8a** 89% **8b** 97%



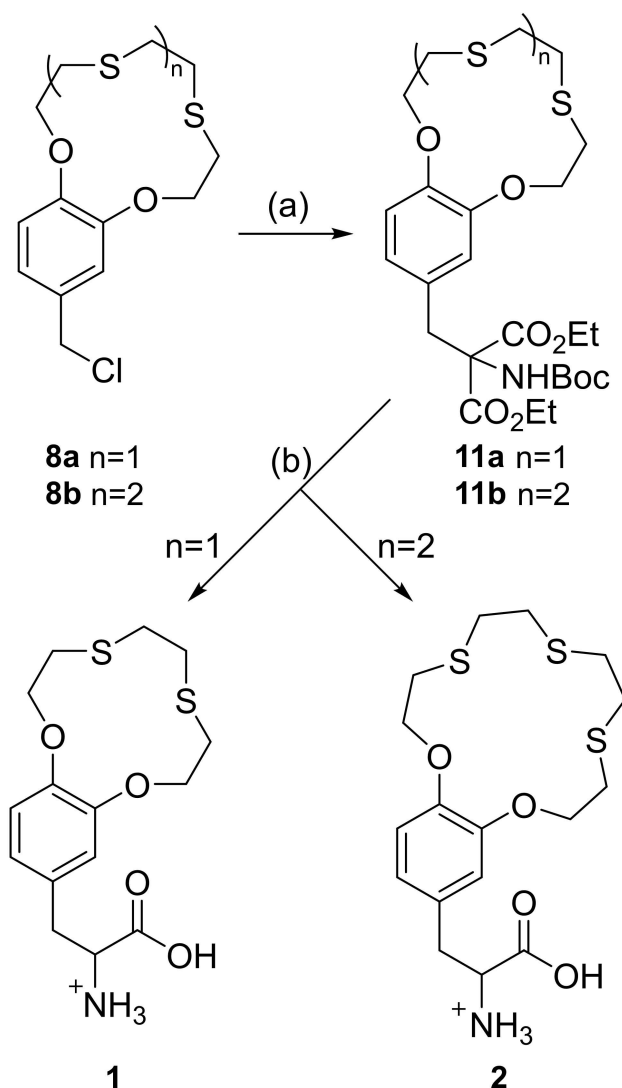
**Scheme 4.** Synthesis of racemic *N*-acetyl amino acids. Reagents and conditions: (a) (I) NaI, acetone (II) *N*-Boc diethylamidomalonate, NaH, DMF, 34% / 52% (b) (I) KOH, H<sub>2</sub>O, EtOH, (II) 0.2 M HCl, 85% / 61% (c) Acylase I, phosphate buffer (d) 32% HCl, reflux

with Acylase I from *Aspergillus melleus*—an enzyme commonly used for racemic resolution of *N*-acetyl amino acids—nor hydrolysis with aqueous HCl could successfully produce the desired amino acids **1** or **2**. With HCl we observed destruction of the crown ether moiety after prolonged treatment, while no reaction was observed with Acylase.

Since removal of the acetyl group proved difficult, we used *N*-Boc diethylamidomalonate as alternative alkylating agent for the chlorides **8a/b** (scheme 5). The resulting *N*-Boc malonates **11a/b** could then be converted into the respective amino acids **1** and **2**. Removal of the Boc group was simultaneously achieved along with decarboxylation.

Thus, we obtained the racemic sulfur containing CEAs in cumulative yields of 9% (**1**) and 17% (**2**).

In addition, we aimed to create CEAs with an amino functionality located in the crown ether moiety. For this purpose, we used the general synthetic strategy outlined in scheme 1a. However, we aimed to suppress the nucleophilicity of the amino group, to prevent the nitrogen-mustard reaction (Scheme 1) from taking place. This suppression can be achieved by reducing the nucleophilicity of the nitrogen atom by using

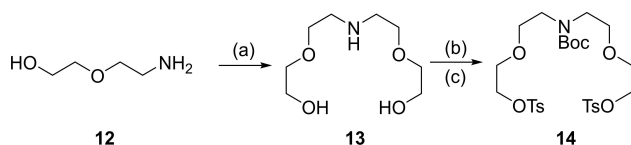


**Scheme 5.** Synthesis of sulfur crown amino acids **1** and **2**. Reagents and conditions: (a) (I) NaI, acetone, (II) *N*-Boc diethylamidomalonate, NaH, DMF, 36% / 67% (b) (I) KOH, H<sub>2</sub>O, EtOH, (II) 0.2 M HCl, 65% / 70%

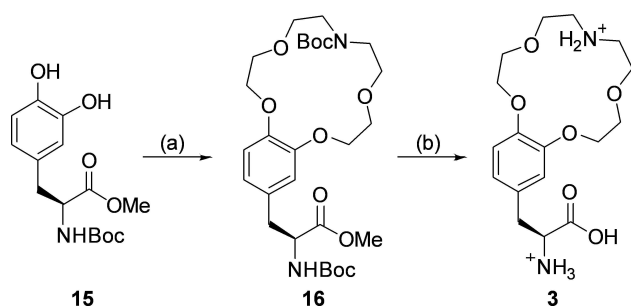
many of the common protection groups for amines. We chose, again, the Boc group, since it reduces the nucleophilicity of the nitrogen. In addition, the Boc group can be cleaved under mild acidic conditions and its cleavage products are easily removed by evaporation.

We prepared a suitable nitrogen-containing precursor molecule according to a procedure from Kang et al., which was slightly modified (Scheme 6).<sup>[19]</sup>

The cyclisation reaction of **14** with DOPA analog **15** was achieved by direct nucleophilic displacement of the tosylate leaving groups with 60% yield to provide the protected CEA **16** (Scheme 7). The Boc group was removed by hydrolysis with aqueous HCl to provide aza-crown ether amino acid **3**. In this case, the crown ether moiety withstood the acidic reaction conditions without fragmentation. The cumulative yield starting from compound **12** was 27%.



**Scheme 6.** Synthesis of alkylation agent **14**. Reagents and conditions: (a) 2-(2-chloroethoxy)ethanol,  $\text{Na}_2\text{CO}_3$ , toluene, 61% (b)  $\text{Boc}_2\text{O}$ , DIPEA, DCM, 85% (c)  $p\text{-TsCl}$ , KOH, DCM, 82%



**Scheme 7.** Synthesis crown ether amino acid **3**. Reagents and conditions: (a) Reagent **14**,  $\text{Cs}_2\text{CO}_3$ , MeCN, 60%, (b) 1 M aqueous HCl, 1,4-dioxane, 90%

## Conclusion

We have achieved the synthesis of the previously unknown sulfur and nitrogen containing CEAs. Due to inability of Acylase I from *A. melleus* to hydrolyze the racemic *N*-Acetyl amino acids **10a** and **10b**, the sulfur containing CEAs were obtained as a racemic mixture with overall yields of 9% (in case of the smaller crown ether **1**) and 17% (in case of the larger structure **2**). Our synthesis, based on the alkylation with ditosylate **4**, avoids the use of compounds structurally similar to mustard gases. Additionally, we prepared a novel nitrogen containing CEAA with 27% yield in 5 steps starting from amino alcohol **12**. The syntheses can easily be executed on a gram scale with standard laboratory equipment. Finally, the synthetic procedures outlined in this work can likely be expanded towards larger crown ether moieties.

## Supporting Information

Experimental procedures, HR-MS and  $^1\text{H}$  as well as  $^{13}\text{C}$ -NMR spectra are provided in the supporting information.

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